SCALE UP OF CYCLONE BIOREACTOR FOR THE PRODUCTION OF BIODEGRADABLE PLASTICS

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ABSTRACT

The production of microbial poly-ß-hydroxybutyrate (PHB) was studied using cyclone bioreactors at both the 1 L lab-scale and in 75 L Pilot Plant Scale (PPS) of Pilot Plant scale. The Culture used for PHB production was *Alcaligenes eutrophus* DSM 545, grown on a mineral salt medium limited by the supply of nitrogen. Two different carbon sources were used: standard commercial glucose and hydrolyzed potato processing waste. The levels of dissolved oxygen obtained in the PPS were strongly depended on the location at which the air was introduced into the reactor. However, with aeration balanced between two injection points and a similar level of power input, 17 W/L, the PPS was able to provide at least as much oxygen transfer capability as the lab-scale reactor. Under all condition tested, the PHB accumulation by *A. eutrophus* was very high, in excess of 80% of the biomass dry weight. The potato starch was readily hydrolyzed to fermentable sugars, however, the economic feasibility of using this carbon sources requires further evaluation.

Keywords : Biodegradable plastics, PHB, cyclone reactor, *alcaligenes eutrophus*

SCALE UP BIOREACTOR CYCLONE SKALA PILOT PLANT UNTUK PRODUKSI PLASTIK BIODEGRADABLE

ABSTRACT

Penelitian untuk menghasilkan poly-ß-hydroxybutyrate (PHB) asal mikroba dilakukan dengan menggunakan bioreactor siklon sekala laboratorium 1 L dan hasil scale-up sekala pilot plant (PPS) 75 L. Kultur *Alcaligenes eutrophus* DSM 545 dibiakan untuk menghasilkan PHB, dengan cara menumbuhkan dalam media garam mineral dengan suplai nitrogen terbatas. Dua jenis sumber karbon digunakan, yaitu: standar glukosa komersial dan glukosa hasil hidrolisis limbah pengolahan kentang. Taraf oksigen terlarur yang diperoleh sangat tergantung dari lokasi dimana udara dimasukkan ke dalam reaktor. Namun, dengan memasukkan udara pada dua tempat dengan tingkat input daya yang sama sebesar 17 W/L, bioreaktor sekala pilot plant mampu untuk memfasilitasi transfer

oxygen secara optimal yang setara seperti yang difasilitasi oleh bioreaktor sekala laboratorium. Berdasarkan hasil pengujian ternyata akumulasi PHB oleh *A. eutrophus* sangat tinggi, lebih dari 80% berat kering biomassa. Pati kentang telah berhasil dihidrolisis menjadi gula yang dapat difermentasi, namun kelayakan ekonomi dari penggunaan sumber karbon asal limbah ini perlu diteliti lebih lanjut.

Kata Kunci: Plastik Biodegradable, PHB, cyclone reactor, *alcaligenes eutrophus*

INTRODUCTION

Bacterial poly-hydroxy-alkanoates, or PHA, is the general term for a class of polyesters containing hydroxyacil monomer units, the most abundant being ß-hydroxybutyrate. A PHA copolymer of hydroxyl-butyrate and hydroxyl-valerate is currently being marketed by ICI under the name Biopol^{TM} as a biodegradable substitute for thermoplastic. In test carried out by Krupp and Jewel (1992), a PHA copolymer was the only so-called biodegradable plastic to show substantial degradation in a bioreactor. PHA can be accumulated intracellularly by a variety of microbes (Anderson and Dawes, 1990), apparently in response to a nutrient limitation (Dawes and Senior, 1973). One of the preferred bacteria is A*lcaligenes eutrophus* , which can provide high yields of polymer from a glucose substrate when subjected to a nitrogen limitation (Senior, 1984). The ICI process for the production of poly-ß-hydroxybutyrate (PHB) using *A. eutrophus* was describes by Byrom (1987). Equipment used included both stirred and airlift bioreactors, with a total fed-batch fermentation time of 110-120 hours resulting in a final PHB accumulation of 75% of the biomass dry weight. Data on the final biomass concentration was not provided. An alternative bioreactor design, referred to as a cyclone column, was describes by Dawson (1974). A cyclone column bioreactor differs from the conventional stirred tank in several respects. The lab-scale cyclone column as illustrated in Figure 1 has a working volume of between 0.8 and 1.5 L. In contrast to the stirred tank, there is no mechanical mixer, instead a pump is used to recirculate the fermentation broth out the conical bottom, trough a side-arm and back into the tangential entry at the top of the reactor. Since the broth is recirculated at high velocity, the tangential entry results in the broth swirling down the inside wall of the reactor.

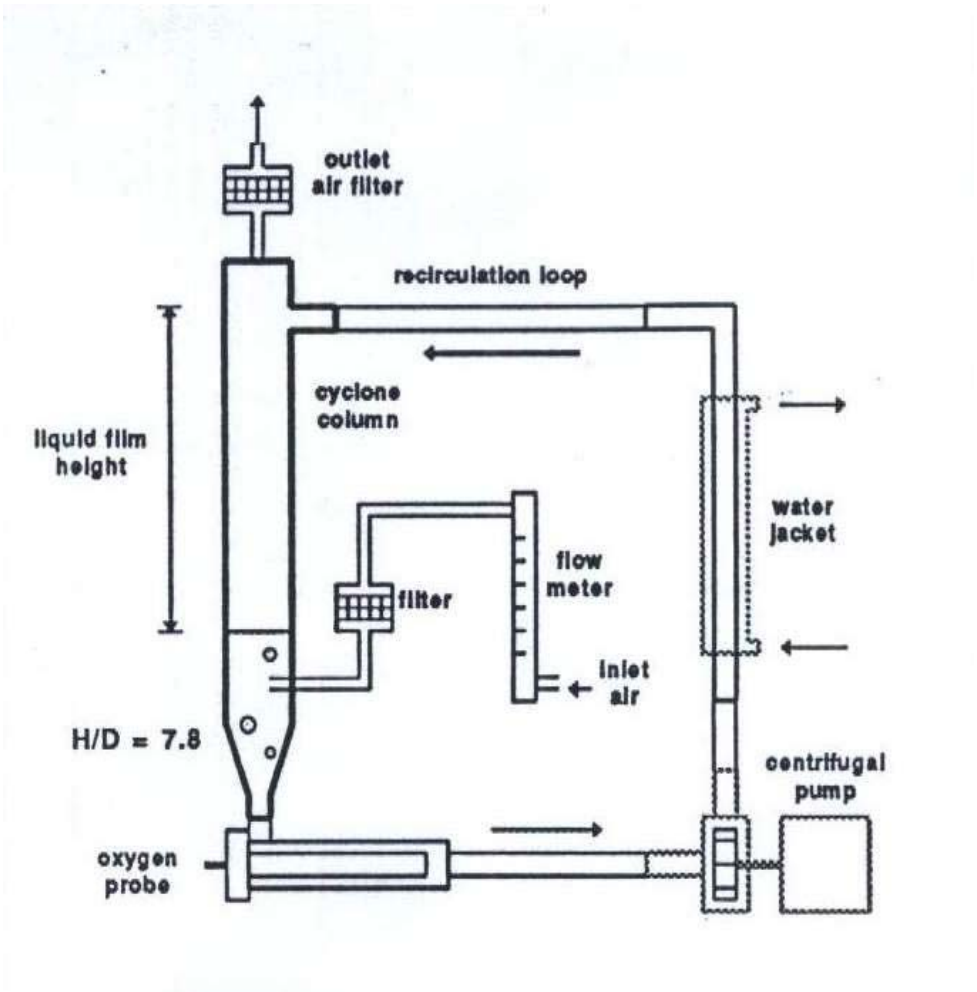


Figure 1. The Laboratory Scale Cyclone Bioreactor

Oxygen transfer from the gas to liquid phase can occur between both of the swirling film and from air bubbles entrained in the recirculation loop. The rate of oxygen transfer is dependent on the film height and is relatively insensitive to changes in the gas holdup from entrained air (Sheppard and Copper, 1990). With a water as the liquid medium a 1.0 L working volume result in a film height of 40 cm corresponding to about 300 mL or 30% of the total liquid volume. The centrifugal pump recirculates the liquid at the rate of 7.9 L/min, corresponding to a residence time in the film of about 1 s and around the whole loop about every 8 s. The lab-scale cyclone requires a higher power input than a stirred tank,

however, the relative simplicity of design allows cyclone reactors to be constructed at lower cost than stirred tanks and, therefore, could help reduce the overall cost for PHB production. This project was undertaken to investigate the use of a cyclone bioreactor for productions of PHB with *Alcaligenes eutrophus* DSM 545.

A 75 L process development unit was designed, constructed and its performance compared to the 1 L reactor. In addition, preliminary tests were performed on a waste from a potato processing plant to investigate the feasibility of hydrolyzing the potato starch to sugars that could be readily utilized by *A. eutrophus.*

MATERIAL AND METHODS

The pilot plant scale

A schematic of the PPS is shown in Figure 2. The PPS consists of a cyclone reactor, progressing-cavity type recirculating pump (Seepex Model 17-12NS), tube-in-tube heat exchanger and various instruments for process monitoring and control as listed in Table 1.

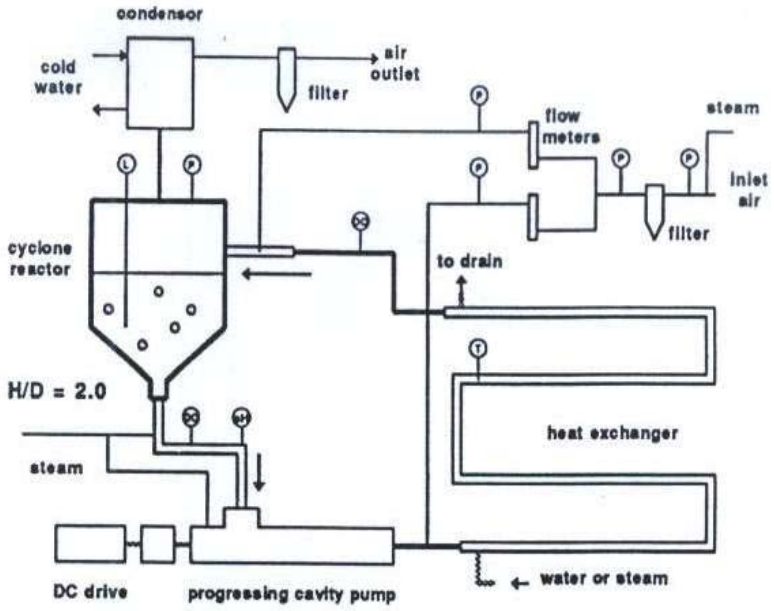


Figure 2. The 75 L Cyclone Reactor Pilot Plant Scale

Table 1. PPS Instrumentation

Function	Instrument	Specifications
Temperature control	YSI Model 2158	Thermistor input ± 0.1^0C ; control to ± 0.5 ^{0}C; two relays for hot and cool water solenoids
pH control	Ingold model 2301 pH Transmitter	Dual relays for acid and base pump; control to ± 0.05 pH units.
Liquid flow	Controlotron System 180	Dual ultra-sonic high temperature heads; 0 to 1,585 L/min ± 1% FS.
Liquid volume	Drexel Brook Eng. Co Series 700	Input from capaciitance level probe model 700-2-24;38 to 130 L ± 1% FS.
Air flow	Cole-Parmer Model 32603-00 meter	0.5 to 4.0 scfm (14-113 L/min) ±10% FS; steam sterilizable
Air pressure	Weksler Model AA44-2 Gauge	0 to 60 psig (0-414 kPa) ±0.5% FS
Dissolved oxygen	Ingold Model 170 % air amplifier	Input from Ingold 120mm polarographic sensor; 98% response in 20 s at 37^0c;

The cyclone reactor and heat exchanger were custom made from 316 stainless steel by Triton Engineering Ltd. (Halifax, N.S.) and Docal Ltd (Montreal, Quebec) respectively. The specifications of the two system, lab-scale and PPS, are compared in Table 2.

Table 2 Cyclone Column and PPS Comparison

Feature	Lab-scale Cyclone	PPS
Working liquid volume (usual)	0.5 to 2.0 (1.0)	40 to 140 (75)
Pump capacity (L/min)	7.9 fixed speed	50 to 125 Variable Speed
Pumping power (Watts)	14	400 to 1,700
Aeration (L/min)	0.5 to 1.0	30 to 135
Height/diameter ratio	7.8	2.0
Entrance velocity	1.0	0.41 to 1,0
Recirculation loop -length (m) -volume (L) -residence time(s)	 0.6 0.1 0.8	 22.9 26 12 to 31

The total working volume of the PPS can be varied between 10 and 130 L, resulting in a scale-up factor of about 100. The recirculating pump has a variable speed 7.5 kW D.C. drive motor. With a pump speed of 350 RPM, the recirculation rate is 118-L/min. Thus with a 75 L working volume the broth is recirculated about 1.6 per minute or every 38 s. Under these conditions the power requirement is 1.3 kW or 17 W/L. Whereas at 200 RPM, the recirculation rate is 90L/min and the power requirement is only 8 W/L.

The two significant design differences between the lab-scale reactor and the PPS are the height to diameter ratios of the cyclones and the relative residence times of the broth in the recirculation loops. With a height to diameter ratio of only 2 in the PPS, oxygen transfer across the swirling gas-liquid interface will be minimal compared to the lab-scale cyclone with a height to diameter ratio of 7.8. Therefore, oxygen transfer must rely more on bubbles entrained in the recirculation loop.

In the PPS the recirculation loop is constructed of 5 cm (2 inch) diameter schedule 40 pipe on the suction side if the pump, and 2.5 cm (1 inch) diameter

schedule 40 pipe on the discharge side, which includes the tube-in-tube heat exchanger. The total volume of the recirculation loop is 26 L, or about 30% of the normal working volume. With a 200 RPM pump speed and a flow rate of 90 L/min the broth spends about 17 s on recirculation loop of the PPS, compared to less than one second in the side-arm of the lab-scale reactor. Thus, the low aspect the ratio of the reactor and long recirculation loop necessitate more than one air injection point in the PPS. There are two points of air injection as shown in Figure 2: just before the broth enter the cyclone (primary aeration), and at the pump discharge (secondary aeration).

Polyhydroxybutyrate production.

Inocula were prepared by transferring a culture of *Alcaligenes euthropus* DSM 545 from nutrient agar plates to 500 mL shake flasks containing 100 mL of mineral salts medium. The medium was limited by nitrogen therefore, changing the concentration of di-ammonium sulphate changed the final biomass concentration that could be achieved. The flakes where shaken at 30°C for about 24 hours and then the culture was aseptically transferred to sterilized lab-scale cyclone. During the lab-scale experiments, excess phosphate in the medium buffered the pH to between 7.0 to 1.5 , whereas, control of pH in the PPS was accomplished using an Ingold Model 2301 pH Transmitter, Ingold pH electrode Model 405-DPAS-K8S/200 and a Masterflex peristaltic pump adding 2 N NaOH. Sterilization of the lab-scale cyclone was achieved by simply placing the entire reactor, side-arm, connecting tubing, pump head and air filters into an autoclave for 30 minutes at 121°C. The growth medium was sterilized in these separate vessels in autoclave at 121°C, cooled and then transferred aseptically into the reactor before addition of the inoculum. Sterilization of the PPS was more complex, consisting of two stages. The first stage was performed by with the reactor empty. Steam was sparged in to the system at two points: in through sparging for 20 minutes, the steam supply was closed and the filters were cooled to ambient temperature with about 10 L/min of air. After completion of this stage, the air lines were isolated from the reactor by closing ball valves. The reactor was then filled with 70 L of water, the recirculating pump was started, an appropriate amount of glucose powder was added and steam was introduced by in to the outside tube of heat exchanger. The composition of the pump stator, Buna-N, limited the sterilization temperature to a maximum of 95°C. This temperature was maintained by regulating the rate of steam supplied to the heat exchanger. After one hour the steam was replaced by cooling water, regulated by a YSI Model 2158 controller to maintain the fermentation temperature at 30±0.5°C. The mineral salt solutions were sterilized as two separate 2 L fraction, cooled and then aseptically pumped into the reactor to obtain a working volume of 74 L addition of the gave a final volume of 75 L.

Analyses.

Samples from the bioreactors were analyzed for biomass dry weight, ammonia nitrogen using an Orion specific ion electrode Model 95-10, glucose using the DNS method (Miller, 1959), and poly-ß-hydoxybutirate using the method of Riis and May (1988) with a Hewlett-Packard Model 5890 Gas Chromatograph. Dissolved oxygen was measured at the exit from the cyclone reactor using an Ingold sensor Model 4176133820, and an Ingold amplifier Model 170, connected to an Asea Brown Bovery chart recorder Model SE 120.

Starch Hydrolysis.

Settled solids with about 55% moisture content were obtained from a potato processor (Humpty Dumpty Ltd., Lachine, Quebec). Hydolysis of starch solid to sugars was accomplished in a two step enzymatic process; The first step used the enzyme alpha-amylase from *Aspergillus oryzae* (Sigma Chemical Co., St. Louise) at 70°C for 30 minutes, follwed by the addition of amyloglucosidase from *Rhizopus* mold (Sigma Chemical Co., St. Louise) at 55°C for 170 minutes. A phosphate buffer maintained the pH in the first step at 6.65, while the second step required pH adjustment to 5.2. Analysis of the free glucose was performed with an enzymatic assay (Sigma Diagnostics, Cat. No. 510-A).

After hydrolysis was completed the solution was centrifusged to remove any remaining solids and sterilized in an autoclave at 121°C for 20 minutes. An appropriate quantity was then added to the mineral salts solution to provide a final glucose concentration of about 15 g/L. The medium was then tested for its suitability as a growth medium for *A. eutrophus* in the lab-scale cyclone bioreactor.

RESULTS.

Lab-scale cyclone.

Figure 3 Illustsrates the results from a typical batch production experiment in the lab-scale cyclone reactor. The initial nitrogen supply of 0.12 g/L was exhausted after about 14 hours, corresponding to 1.9 g/L of biomass with 30% PHB content. At this point 3.1 g/L of glucose had been consumed, resulting in a biomass yield based on glucose of 0.61.

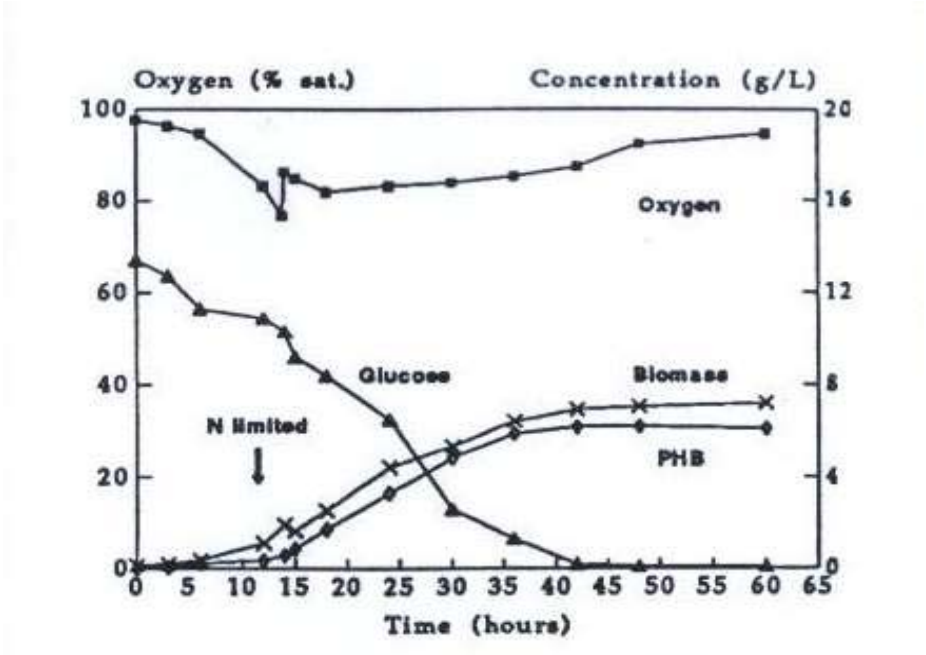


Figure 3. Results from the batch growth on *A. eutrophus* in a 1 L cyclone reactor on a glucose and mineral salts medium.

During the subsequent 50 hours PHB accumulation continued under nitrogen limited conditions. The glucose was exhausted after a total time 48 hours, resulting in a final biomass concentration of 7.1 g/L with 87% PHB content. This gives an overall yield from glucose of 0.53 and 0.46 for total biomass and PHB respectively. This is very close to the maximum theoretical yield calculated for PHB from glucose of 0.46 (Yamane 1993). The minimum concentration of dissolved oxygen, 78% of saturation,occurred just prior to nitrogen limitation. During the majority of the PHB accumulation phase, the concentration of dissolved oxygen averaged between 82% and 87% of saturation. The kinetics for both growth and PHB production are very similar to those reported by Sonnleitner *et al* (1979) for A*. eutrophus* H 16 growing on lactate as a carbon source in a conventional stirred rector, although only 15% PHB based on dry weight was achieved during growth, with a maximum accumulation of 78% PHB.

Process development unit

Three experiments were performed using a 75 L working volume. The results are summarized and compared to results obtained in lab-scale cyclone in Table 3.

Table 3. Summary of PHB Production

Reactor	PHB conc. (g/L)	PHB Yield (g/g glucose)	% of Biomass	Aeration[1] (vvm)	Power[2] (W/L)	Min. D.O. (% sat.)
Lab-scale	6.2	0.46	87	0.5	14	78
PPS	2.0	0.28	80	0.76/0.0	8	0
PPS	2.4	--	89	0.0/1.4	8	74
PPS	5.0	0.38	96	0.2/0.4	17	84

[1]For the PPS the data refers to both primary/secondary aeration
[2]Data refers to electrical power required for pumping

The three pilot–scale experiments differed by the amount and place aeration, pump speed and initial concentration of nitrogen in the growth medium. In the first and second experiments 0.28 g/L of $(NH_4)_2SO_4$ were used, with a pump speed of 200 RPM and either 57 L/min of primary aeration or 107 L/min of secondary aeration. Whereas, the results from the third experiments were obtained with approximately 0.84 g/L di-ammonium sulphate and a higher pump speed, 350 RPM, but with reduced aeration, a total of 45 L/min split between the primary and secondary aeration points.

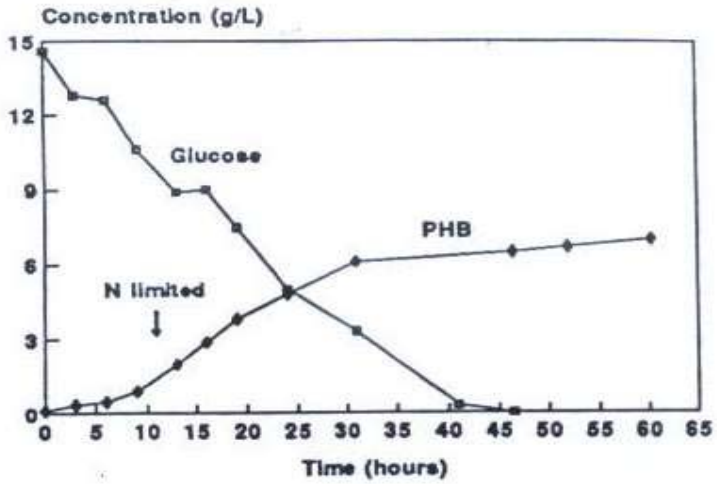
The inoculum for PPS was obtained from the 1 L cyclone and was, therefore, 1.3% compared to 10% by volume when the small cyclone was inoculated from a shake flask. In the pilot-scale experiments the growth kinetics were similar to those obtained in lab-scale cyclone, with the nitrogen being exhausted between 15 and 20 hours.

Accumulation of PHB continued for an additional 40 to 45 hours or until the time of glucose exhaustion. The final biomass concentrations in the first two experiments were 2.5 and 2.65 g/L, with PHB contents of 80% and 89% respectively. In the third experiment with a higher initial nitrogen concentration, the final biomass and PHB concentrations were 5.1 and 4.9 g/L.

Although in the PPS experiments the accumulation of PHB as percentage of biomass dry weight remained very high, the yield of PHB based on total glucose consumed decreased to between 0.28 and 0.38, significantly less than the 0.46 obtained in the lab-scale cyclone.

Potato starch hydrolysis

The two stage enzymatic hydrolysis process resulted in approximately 90% of the theoretical yield of glucose. Thus, with 84% of the dry solids as carbohydrate, the final glucose concentration obtained was 400 g/L. After dilution to 15 g/L with the mineral salts solution, the solution was inoculated with *A. eutropus*. The results are illustrated in Figure 4. The kinetics of growth and PHB accumulation were very similar to those obtained with the standard glucose solution and the overall yield of PHB was at least as high, approaching the theoretical maximum.



DISCUSSION.

The results have provided some preliminary information with respect to both the scale-up criteria for cyclone bioreactors and the performance of these reactors for the production of PHB using *A.eutropus.* The low aspect ratio of the 75 L cyclone and long recirculation loop have a significant effect on the concentration of dissolved oxygen that can be maintained without using secondary air injection. With the pump speed of 350 RPM and recirculation rate of 118 L/min, the broth spends only about 20 s in the loop. This was adequate to maintain in excess of 84% saturation of dissolved oxygen with 5 g/L of biomass.

The use of primary air alone combined with a moderate pump speed of 200 RPM results in limited gas holdup and is impractical for high cell densities. Gas holdup can be increased by increasing, either the pump speed or secondary aeration at the pump discharge. Increasing the pump speed results in a lower retention time in the cyclone reactor and greater liquid velocity and turbulence in the recirculation loop, albeit with increased power consumption. Increased secondary aeration also requires more power and beyond 35 L/min does not significantly improve gas holdup, except a low pump speeds when no primary aeration was used. Therefore, assuming that the aeration is judiciously divided between the cyclone and the loop, it is apparent that the most important scale-up criterion is the power input to the recirculation pump. The power requirements will vary depending on the concentration of biomass and it will require further study to characterize this relationship.

The kinetics of PHB production were similar to those reported for *A. eutropus* growing in a conventional stirred tank (Sonnlitner *et al* 1979), except for the high PHB levels whn expressed as a percentage of biomass dry weight. Final PHB

levels were at least 80% of the biomass dry weight and in one experiment reached 96%. In addition, there were always significant levels of PHB present before nitrogen limitation occurred. It has been previously demonstrated that *A. eutrophus* is influenced by the concentration of dissolved oxygen, for both controlling respiration rate during growth (Sonnlitner *et al.* 1979) and affecting the synthesis of PHB (Vobrecht *et al*. 1979,Steinbuchel and Schlegel 1989, Steinbüchel and Pieper 1992).

Cyclone bioreactors differ from conventional stirred tanks with respect to the agitation and the oxygen transfer characteristic. Unlike the uniform mixing in a stirred tank, the method of using a pumped recirculation loop for achieving agitation results in plug-flow hydrodinamics. The culture is continuously cycled through variations in both the preassure and concentration of dissolved oxygen, with a frequency of cycling determined by the pumping rate relative to the total working volume.

In the lab-scale cyclone the cycling frequency was about 7.5/min, while in the PPS the cycling frequency varied between 1.6 and 1.2/min. The cycling frequency, as well as the aeration, would have an effect on the magnitude of changes in the dissolved oxygen concentration. The higher the frequency, the closer the agitation will approach perfect mixing with correspondingly less change in the level of dissolved oxygen. Therefore, a possible explanation for the high PHB percentages is the extra stress imposed on the cells as results of fluctuations in the oxygen supply. However, these results require further investigation before definitive explanation can be provided.

CONCLUSIONS

1. The levels of dissolved oxygen obtained in the PPS were strongly depended on the location at which the air was introduced into the reactor.
2. Aeration balanced between two injection points and a similar level of power input, 17 W/L, the PPS was able to provide at least as much oxygen transfer capability as the lab-scale reactor.
3. The PPS Using pump speed of 350 RPM and recirculation rate of 118 L/min, the broth spends only about 20 s in the loop. This was adequate to maintain in excess of 84% saturation of dissolved oxygen with 5 g/L of biomass.
4. The PPS has proved to produce a high percentage of PHB, in excess of 80% of the biomass dry weight. However, the result requires further investigation prior to definitive explanation can be provided.
5. The enzymatic hydrolysis of potato processing waste to produce fermentable sugars is definitely technically feasible. High conversions were obtained in the starch hydrolysis, followed by good growth and PHB accumulation by *A. eutropus.* However, the economic feasibility of replacing conventional sugars sources with the potato waste has not been evaluated. This requires further optimization of the hydrolysis time.

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